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14. ABSTRACT
The overarching goal of this work was to develop a computational modeling approach for predicting uptake of inhaled hydrocarbon (HC) aerosols and vapors from petroleum-based and synthetic hydrocarbon mixtures. Using a novel approach for measurement of aerosolized HC uptake and distribution that was developed for n-tetradecane, and data collected from exposure to HC mixtures and jet fuels, physiologically-based pharmacokinetic (PBPK) models were developed for aliphatic and aromatic jet fuel constituents, or "markers." Published vapor exposure models were also modified to describe deposition and uptake of aerosols. The remaining fuel mass was divided into "lumps" of unspeciated constituents. Three lump models were constructed; an aromatic constituent lump, a mid-range aliphatic lump, and a high molecular weight aliphatic lump. Competitive inhibition of metabolism was assumed to occur with 2 of three lumps and most of the marker hydrocarbons. The PBPK fuel model can account for exposure to vapor-only and aerosol+vapor fuel atmospheres. This model is the first jet fuel model that can be used for dose response analyses and in risk assessment . Additionally, partition coefficients were determined in vitro for a series of C9 isomers found in HC mixtures, and for additional aromatics in neat or aerosolized jet fuel for use in future HC models.

15. SUBJECT TERMS
Computational Modeling, mixtures, SPME-GC/MS, TDS-GC/MS, PBPK, petroleum fuels, inhalation

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Final Report
Computational Approaches for Predicting Nonlinear Interactions of Chemical Mixtures in Biological Systems

For Dr. Walter Kozumbo

December 2009
Final Report for: 1 February 2007 to 30 November 2009

Grant Number: FA9550-07-1-0132

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EXECUTIVE SUMMARY

This report will summarize key points of the published and unpublished research undertaken during this grant and describe the importance of the collected data and resulting computational models to the study of complex mixtures.

Over the course of the reporting period, partial or full support was provided for one MS student, one MPH Student, one PhD student, as well as two undergraduate students, and one post-doctoral scientist. The personnel were Ghanashyam Joshi, Anping Chang, Sheppard A. Martin, Kristyn F. Brunson, Christine Kendrick, and Raphael Tremblay. The PhD dissertation and MS thesis for two of the graduate students may be found in pdf format after May 2010 at the University of Georgia web site below. The MPH capstone project and undergraduate student internship project reports are available upon request to the PI for this work. After May 2010 to access the PhD dissertation and MS thesis, type the student name in the space provided at the website and follow directions for Sheppard A. Martin and Ghanashyam Joshi.

http://dbs.galib.uga.edu/cgi-bin/ultimate.cgi?dbs=getd&userid=galileo&action=search&cc=1

Background and Significance

Jet fuels are complex hydrocarbon (HC) mixtures composed of hundreds of different chemicals with the potential for metabolic interactions and perhaps other interactions that affect the toxicity of the fuel. The neurotoxicity, immunotoxicity, and respiratory toxicity of fuels such as JP-8 have been described in the published literature, however assessment of fuel pharmacokinetics is found in only a few publications. The overarching goal of this work was the development of a physiologically-based pharmacokinetic model for complex HC mixtures, such as jet fuels, that was capable of predicting the tissue pharmacokinetic behavior resulting from exposure to jet fuels at multiple concentrations and in both aerosol and vapor form. While the chemical composition of liquid jet fuel (JP-8 and S-8) has been well described, there is a paucity of atmosphere characterization of inhaled fuels; a necessity for the development of a PBPK model. Lack of exposure characterization to complex mixtures of fuel vapor or vapor plus aerosols created the task for us to develop analytical methods to measure the chamber fuel atmospheres. Historically chamber concentrations of fuel were reported as total mass of fuel per cubic meter of air. To interpret the published toxicology data and develop a computational strategy to describe the dosimetry of inhaled jet fuel hydrocarbons, it was imperative that we: 1) develop an inhalation exposure system for the adult rat, 2) develop methods to characterize the chemical composition and characteristics of the exposure atmosphere, and 3) develop analytical methods to measure low levels hydrocarbons in tissues for time course data sets.

During the first, and part of the second year, of the grant period our focus was on completing the above three goals. Following completion of these first projects, we began the development of computational models to describe the kinetics of chemicals present in

JP-8 and S-8, in particular n-tetradecane and n-octane. These initial models represented the first steps in development of a PBPK model for jet fuel, aiding in development of a computational strategy to assess aerosol droplets and vapors. The jet fuel PBPK model describes the adsorption, distribution, metabolism, and excretion of aromatic and aliphatic jet fuel constituents in both aerosol and vapor form. This fuel PBPK model is also applicable for other complex mixtures of military and civilian interest, such as gasoline, diesel, white spirit, and kerosene, provided the exposure atmosphere is well characterized. To further our understanding of the kinetic behavior of relevant HCs, additional preliminary PBPK models were developed to describe the kinetic behavior of individual n-alkanes and mixtures of n-alkanes commonly found in fuels and other HC mixtures.

Published Abstracts and Presentations

Martin SA, Tremblay RT, **Fisher JW**. (accepted 2009) Progress in the Development of a Physiologically-Based Pharmacokinetic Model for Aviation Fuels. 49th National Meeting of the Society of Toxicology, Salt Lake City, UT. Abstract (in press), March 2010.

Joshi, G, Tremblay RT, **Fisher JW**. (accepted 2009) Tissue Partition Coefficients For Nonane and its Isomers. 49th National Meeting of the Society of Toxicology, Salt Lake City, UT. Abstract (in press), March 2010.

Fisher JW, Tremblay RT, Mattie DR, Martin SA. (submitted 2009) PBPK Modeling and Kinetics of JP-8 and S-8. Presentation at Annual Toxicology and Risk Assessment Conference. West Chester (Cincinnati), OH April 27-28 2010.

Martin SA, Kendrick C, Flynt K, Tremblay RT, **Fisher JW**. (2009) Development of a Rat PBPK Model for a Prominent n-Alkane, Tetradecane, found in Aviation Fuels JP-8 and S-8. 472, *The Toxicologist CD — An official Journal of the Society of Toxicology*, 102(S-1).

Tremblay RT, Martin SA, **Fisher JW**. (2009) Evaluation of Fecal and Urinary Excretion Products After Inhalation of Aerosolized S-8 Synthetic Jet Fuel in Rats. 953, *The Toxicologist CD — An official Journal of the Society of Toxicology*, 102(S-1).

Kendrick CM, Martin SA, **Fisher JW**, Adams TT, Tremblay RT. (2008) Evaluation of Potential Inhalation Hazards of Petroleum-, Synthetic- and Bio-Fuels Using GC/MS Analysis of Vapors under Equilibrium Conditions. 1464, *The Toxicologist CD — An official Journal of the Society of Toxicology*, 102(S-1).

Martin SA, Kendrick C, Flynt K, Tremblay RT, **Fisher JW**. (2008) Inhalation Kinetics of Jet Fuel Components in the Rat. 948, *The Toxicologist CD — An official Journal of the Society of Toxicology*, 102(S-1).

Tremblay RT, Martin SA, **Fisher JW**. (2008) A Novel Method for the Chemical Characterization of Generated Jet Fuel Vapor and Aerosol for Animal Studies. 1100, *The Toxicologist CD — An official Journal of the Society of Toxicology*, 102(S-1).

Kendrick CM and **Fisher JW**. (2008) Evaluation of Potential Inhalation Hazards of Petroleum-, Synthetic- and Bio-Fuels Using GC/MS Analysis of Vapors under Equilibrium Conditions. In: The Honors Program's Center for Undergraduate Research Opportunities 2008 Symposium Program and Book of Abstracts. University of Georgia. [Online]: http://www.uga.edu/honors/forms/current_students/curo/symp/bk_of_abstracts/book_abstracts_2008.pdf

Campbell JL and **Fisher JW**. (2007) PBPK Modeling Assessment of the Competitive Metabolic Interactions for m-Xylene, Ethylbenzene, and a Lumped Aromatic Fraction of JP8 Jet Fuel Vapor. 1688, *The Toxicologist CD — An official Journal of the Society of Toxicology.*

Anand SS, Campbell JL, and **Fisher JW**. (2007) In vitro Hepatic Metabolism of n-Alkanes, n-Nonane, n-Decane, and n-Tetradecane. 960, *The Toxicologist CD — An official Journal of the Society of Toxicology.*

Peer reviewed manuscripts (as of December 2009)

Martin SA, Kendrick C, Brunson KF, Tremblay RT, **Fisher JW**. (2010) Characterization of a Nose-Only Inhalation Exposure System for Hydrocarbon Mixtures and Jet Fuels. *Inhalation Toxicology* (in press).

Tremblay RT, Martin SA, **Fisher JW**. (2010). Novel Characterization of the Aerosol and Gas Phase Composition for Aerosolized Jet Fuel Atmospheres. *Inhalation Toxicology* (in press).

Manuscripts in Preparation (with tentative titles)

Martin SA, Tremblay RT, **Fisher JW**. (200x) Development of a Physiologically-Based Pharmacokinetic Model for Complex Hydrocarbon Mixtures: Aviation Fuels.

Martin SA, Tremblay RT, **Fisher JW**. (200x) A Novel Physiologically-Based Pharmacokinetic Modeling Strategy for Aerosol and Vapor Exposures to n-Alkanes in the Rat: C6-C15.

Joshi, G, Tremblay RT, **Fisher JW**. (200x) Tissue Partition Coefficients For Nonane and its Isomers.

Tremblay RT, Martin SA, Fisher JW. (200x) Determination of the Metabolic Profile Resulting from Exposure to Synthetic Jet Fuel (S-8) in the Rat.

Book chapter

Tremblay RT, Martin SA, **Fisher JW**. (2010) Evaluation of Methods Used to Generate and Characterize Jet Fuel Vapor and Aerosol for Inhalation Toxicology Studies. In: Jet Fuel Toxicology. Eds: Mark Witten, Glenn Ritchie, and Errol Zeiger. *In press* July 2010.

Collaborations/Consultations/Interactions

Collaboration
Laurence D. Fechter, PhD Loma Linda Veterans Administration Medical Center, Loma Linda, CA

Contributions

2008 – Collaboration includes use of our novel exposure atmosphere sampling technique (Tremblay et al, in press) to characterize the inhalation exposure chamber for JP-8 and S-8 exposures at Loma Linda Veterans Administration Medical Center in the laboratory of Dr. Fechter. We also conducted analysis of exposed rat tissues collected from inhalation chambers on site following exposure to fuel and/or noise using our improved tissue analytical method, described in abstract form and presented at the 2008 SOT meeting (Martin, et al, 2008) and to be submitted for journal publication as part of a PBPK modeling paper in 2010. The method was a modification of our earlier publication "Campbell, JL Jr and **Fisher JW**. (2007) A PBPK modeling assessment of the competitive metabolic interactions of JP-8 vapor with two constituents, m-xylene and ethylbenzene. Inhal Toxicol. Mar;19(3):265-73. Data sets will be used for mixtures modeling.

Collaboration

David R. Mattie, PhD. AFRL/HEPB**,** Wright-Patterson Air Force Base, Ohio

Contributions

2007-2009 – Collaboration includes continual interaction and project support during the grant period, as well as assistance in receipt of jet fuels for analysis and exposures.

Interaction
Joint meeting and site visit by Dr. Walter Kozumbo to assess the status of current jet fuel related research conducted at the University of Georgia (UGA), Athens, GA and Wright Patterson Air Force Base (WPAFB), Dayton, OH.

Contributions
2008 - Active discussion and debate on the progress and status of fuel research at UGA and WPAFB with special emphasis on inhalation work and respective PBPK modeling efforts at UGA and WPAFB, novel atmospheric and tissue (lung and feces) analytical methods developed at UGA, suggestions for data sharing and future work by Dr. Walter Kozumbo, and discussion of data gaps to be addressed in future research at UGA.

Transitions/Technology Transfers

Transition
Analytical method for detection of chemicals in tissues to David Kim from Syngenta Corporation.

Contribution
2009 - Adaptation of the SPME-GC/MS method for measuring tissue:blood partition coefficents.

Transition

Transition of kinetic data to David R. Mattie, PhD. AFRL/HEPB, Wright-Patterson Air Force Base, Ohio

Contribution
2009 - Transition of kinetic tissue data to David Mattie, PhD, (Wright Patterson Air Force Base, Dayton, OH) occured under co-operative agreement between the University of Georgia and WPAFB. Transition of data will improve PBPK modeling capabilities through cooperative discussion and the individual modeling efforts of personnel from both research groups for JP-8, S-8, and 50:50 blend of JP-8:S-8.

Transition
Transition of kinetic data to Harvey J. Clewell, PhD and Jerry L. Campbell, Jr. Ph.D. The Hamner Institutes for Health Sciences, Research Triangle Park, NC

Contribution
2007 – Transition of tissue pharmacokinetic datasets for n-alkane/PAH (naphthalene) mixture exposure in the rat to The Hamner Institutes for Health Sciences (Research Triangle Park, NC), to support development of non-cancer toxicity factors for naphthalene within US EPA.

Honors or Awards received while supported by AFOSR (2007-2009)

Outstanding Leadership Award. (2009). Awarded to Sheppard A. Martin by the Society of Toxicology, Student Advisory Council. March, 2009.

Best Abstract Award. (2008). Awarded by Society of Toxicology, Mixtures Specialty Section, for:

> Martin SA, Kendrick C, Flynt K, Tremblay RT, **Fisher JW**. Inhalation Kinetics of Jet Fuel Components in the Rat. 948, *The Toxicologist CD — An official Journal of the Society of Toxicology*, 102(S-1).

2nd Place Award. Poster Presentation (2009). Awarded by University of Georgia Interdisciplinary Toxicology Program Symposium, for:

Martin SA, Kendrick C, Flynt K, Tremblay RT, **Fisher JW**. (2009) Development of a Rat PBPK Model for a Prominent n-Alkane, Tetradecane, found in Aviation Fuels JP-8 and S-8.

John J. Sheuring Scholarship (2008-2009) awarded to undergraduate student Kristyn Flynt.

John J. Sheuring Scholarship (2007-2008) awarded to undergraduate student Christine Kendrick.

Best Student Abstract/Poster Award. (2007) Awarded to Jerry L. Campbell, Jr. by the Society of Toxicology Biological Modeling Specialty Section, for:

Campbell JL and **Fisher JW**. PBPK Modeling Assessment of the Competitive Metabolic Interactions for m-Xylene, Ethylbenzene, and a Lumped Aromatic Fraction of JP8 Jet Fuel Vapor. Abstract. 2007.

Key Findings/Results/Accomplishments

Experimental Objectives:

Method Development

- Design and characterize a nose-only inhalation exposure system for use in exposures to hydrocarbon droplets and vapors.
- Improve characterization of vapor/aerosol phase hydrocarbon mixtures in exposure atmospheres.
- Characterization of JP-8, S-8, 50:50 JP-8:S-8, diesel, biodiesel, biomass-fuel, biodiesel/biomass-fuel blend headspace vapor via GC/MS
- Improve our detailed tissue characterization method to support PBPK model development
- Obtain detailed understanding of the clearance behavior (metabolism and elimination) of marker hydrocarbons in JP-8 and S-8.

Animal Exposures

- Collect kinetic data following exposure to a custom mixture of eight hydrocarbons, for use in the development of a hydrocarbon mixture PBPK model (Table 1).
- Exposures to complex hydrocarbon mixtures JP-8, S-8, and JP-8:S-8 (50:50 blend). Collect kinetic tissue data for development of hydrocarbon PBPK models.
- Exposures to n-tetradecane (C14), as a high molecular weight (predominately aerosol phase) chemical marker of aviation fuel exposure, and n-octane (C8), as a low molecular weight n-alkane (predominately vapor phase) marker, for development of PBPK models for fuel constituents.

Model Development

- Develop a PBPK model for aviation fuels JP-8 and S-8
- Develop PBPK models for n-alkane constituents of aviation fuels and other complex hydrocarbon mixtures

Closing Data Gaps

- Investigation of potential blood cell binding by aviation fuel hydrocarbons
- Fill data gaps in the partition coefficient (PC) literature related to PBPK model development. Specifically, collect lung PC values for marker chemicals where data is lacking in the current literature.
- Determine partition coefficients for a series of isomers of n-nonane, a prominent synthetic and petroleum-based jet fuel constituent in both aerosol droplets and fuel vapor.

Table 1: Summary of Exposures Conducted During the Grant Period

Chemical(s)	Concentration (mg/m3, ppm*)	Expos. (hrs)	Time Points (hrs)
Designer Mix A – High	1270	4	2, 4, 4.5, 5, 6, 8
Designer Mix A – Low	630	4	2, 4, 4.25, 4.75, 5.25, 6
Designer Mix A – Fat	540	4	4, 16, 28, 42, 76, 100
Tetradecane (C14)	90	4	2, 4, 4.25, 4.75, 5.25, 6
S-8	1060	4	2, 4, 4.25, 4.5, 4.75, 5, 28, 52
JP-8	900	4	2, 4, 4.25, 4.5, 4.75, 5
Designer Mix B-JP-8/S-8 Blend	200	4	2, 4, 4.17, 4.5
Octane (C8) vapor-High	5000*	2	2
Octane (C8) vapor-Med	1000*	2	2, 2.08, 2.33, 2.66
Octane (C8) vapor-Low	100*	2	2

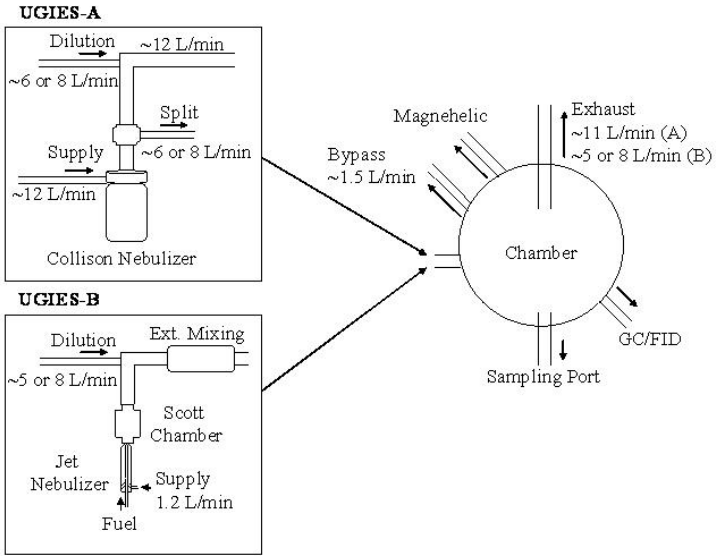
Method Development

Design and Characterization of a Nose-Only Inhalation Exposure System

To support development of physiologically-based pharmacokinetic (PBPK) models for complex hydrocarbon mixtures, such as jet fuels, it was imperative that we obtain a detailed understanding of the atmospheric composition of the jet fuel or hydrocarbon mixture exposure atmosphere. There is little data of this type currently published in the literature for JP8 and no data for S-8, or the 50:50 JP-8:S-8 blend of interest to the US Department of Defense. In consideration of the overarching goal of the project, and the type of data required, we developed an inhalation exposure system. The criteria for system development included: 1) Conducive to collection of atmospheric data, such as fuel constituent aerosol and vapor phase composition/concentration and aerosol size distribution, 2) Remain stable during the exposure period, 3) Utility at multiple exposure concentrations and with both aerosolized and vaporized chemicals.

Two generation systems for aerosolizing fuel and hydrocarbons were characterized and implemented for use with jet fuels or a custom eight hydrocarbon mixture (n-decane, n-undecane, n-dodecane, n-tridecane, n-tetradecane, n-pentadecane, 2-methylnaphthalene, naphthalene) that was of interest for development of a computational modeling strategy for aerosol droplet deposition and uptake (Figure 1). Total hydrocarbon concentration and stability of the HC exposure atmosphere were monitored via online gas chromatograph (GC). Aerosol/vapor (A/V) ratios, and total and individual hydrocarbon concentrations were determined using adsorbent tubes. Tubes were analyzed by thermal desorption-gas chromatography/mass spectrometry (TDS-GC/MS). Droplet size distribution was assessed via 7-stage cascade impactor. Overall, the total A/V hydrocarbon concentrations ranged from ~100-1300 mg/m^3, with between 30 and 80% aerosol content, depending on the mixture. The exposure atmospheres remained stable during the 4-hour exposure periods, with coefficients of variation (CV) of less than 10%. The droplet mass median aerodynamic diameter (MMAD) was between 1-3 µm depending on the generator and mixture utilized. In conclusion, our use of modern instrumentation for the design and characterization of a nose-only exposure system resulted in a vastly improved understanding of the inhalation exposures presented to rodents using both aerosolized fuels and custom hydrocarbon mixtures. This work also provided a platform for collection of pharmacokinetic data used in PBPK model development.

Figure 1: Schematic of Exposure System (From Martin et al, *in press*)



Improve Characterization of Hydrocarbon Mixtures in Exposure Atmospheres

A novel method for characterization of the chemical profile within an exposure system was developed (Tremblay et al, in press). The method utilized commercially available absorbent tubes (Carbotrap 300 thermal desorption (TDS) charcoal tubes (Supelco, St. Louis, MO)) to measure hydrocarbon composition in the vapor and droplets resulting from the aerosolization of jet fuels and hydrocarbon mixtures (Table 2 and Figure 2). The Carbotrap 300 media was selected to allow for later comparison with tissue data and aid in development of PBPK models, based on the tube design which promotes selective adsorption on specific chambers of the tube, and the high throughput capacity afforded by our FY-06 purchased TDS-GC/MS (Gerstel, Inc., Baltimore, MD and Agilent Technologies). Implementation of this method allowed for determination of the aerosol and vapor phase chemical concentrations of 64 hydrocarbons in the jet fuel exposure atmosphere. Additionally, using the differential between measured total (aerosol plus vapor phase) and measured gas phase concentrations, the aerosol and vapor percent, per chemical, were calculated and serve as parameters for PBPK models. All samples were collected for 1 min at 400 mL/min and quantified using thermal desorption gas chromatography mass spectrometry (TDS-GC/MS). The method was validated using 4-hour exposures to aerosolized jet fuels to JP-8 and S-8 at total concentrations ranging from 200 to 1000 mg/m^3. Use of this method during an animal exposure period allowed for development of a time-course for an exposure atmosphere, due to repeated sample collections at ~40 min intervals, resulting in a more detailed picture of the internal chamber environment. By using neat fuel to calculate total concentration and authentic standards to calculate specific compound concentrations comparison to the current exposure data in the published literature is possible. Overall, method accuracy resulted in favorable comparisons to data collected for total concentration using the online GC-FID technique, granted the assessed total concentration of S-8 fuel was more similar than the JP-8 fuel. Individual chemical concentrations in the jet fuel atmospheres remained highly

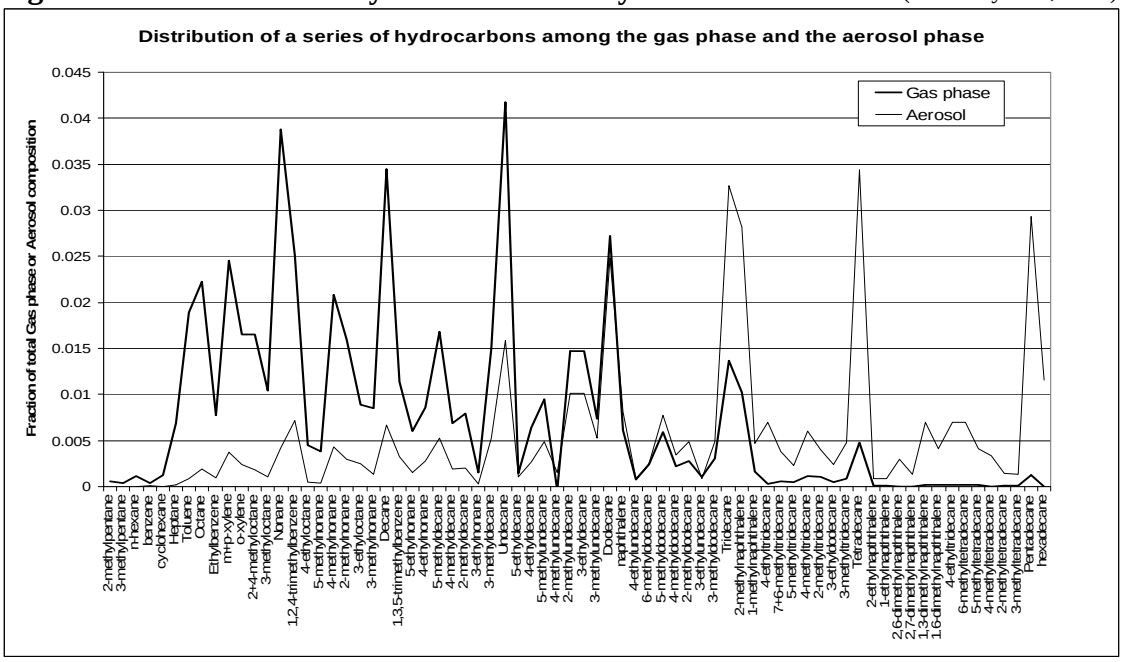
stable, with approximately 15% variability. This method can be used with jet fuels and other complex mixtures for which detailed characterization is necessary.

Table 2. Chamber atmosphere measured concentration (mg/m3) for a series of hydrocarbons from a JP-8 exposure (~900 mg/m3). (Tremblay et al, 2008)

	Gas Phase	Aerosol	Fraction in gas phase
2-methylpentane	0.11	0.00	0.97
3-methylpentane	0.05	0.01	0.87
benzene	0.14	0.03	0.85
2-methylhexane	1.36	0.19	0.88
2,3-dimethylpentane	0.70	0.15	0.82
3-methylhexane	2.01	0.18	0.92
3-ethylpentane	0.03	0.01	0.76
2,5-dimethylhexane	1.07	0.00	1.00
methylcyclohexane	17.24	1.66	0.91
2,4-dimethylhexane	0.69	0.11	0.86
2,3-dimethylhexane	0.71	0.08	0.90
toluene	4.37	0.67	0.87
2-methylheptane	5.59	0.54	0.91
4-methylheptane	1.63	0.16	0.91
3-methylheptane	3.77	0.25	0.94
n-C8	11.81	1.55	0.88
2,5-dimethylheptane	1.67	0.15	0.92
2,3-dimethylheptane	2.37	0.29	0.89
ethylbenzene	2.43	0.54	0.82
3,4-dimethylheptane	0.43	0.13	0.77
2-methyloctane	7.28	0.82	0.90
m-xylene	5.84	1.48	0.80
p-xylene	2.01	0.51	0.80
3-methyloctane	5.81	0.85	0.87
o-xylene	3.82	1.10	0.78
n-C9	14.80	2.44	0.86
2,2-dimethyloctane	0.29	0.17	0.63
isoproprylbenzene	0.88	0.25	0.78
3,3-dimethyloctane	1.45	0.35	0.81
2-methylnonane	4.41	0.97	0.82
1,3,5-trimethylbenzene	1.55	0.66	0.70
3-ethyloctane	0.59	0.14	0.81
3-methylnonane	3.67	0.84	0.81
o-ethyltoluene	2.06	0.87	0.70
1,2,3-trimethylbenzene	2.04	1.17	0.63
1,2,4-trimethylbenzene	5.26	2.64	0.67
butylcyclohexane	1.73	0.52	0.77
4-methyldecane	2.12	0.88	0.71
2-methyldecane	2.90	1.24	0.70
3-methyldecane	3.01	1.30	0.70
n-C10	15.18	4.04	0.79
n-C11	11.27	7.11	0.61

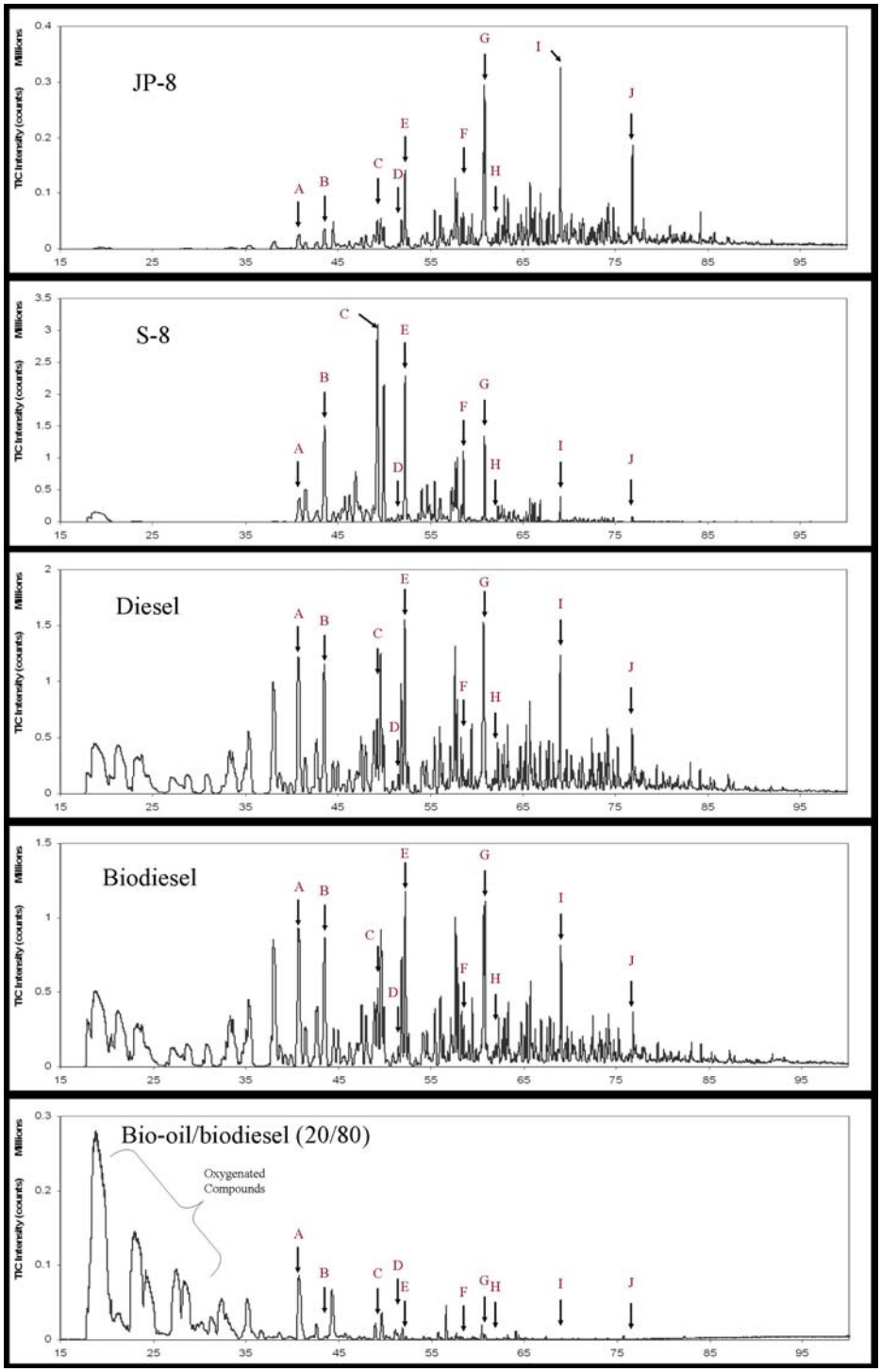
n-C12	6.20	8.25	0.43
Naphthalene	0.12	0.17	0.41
n-C13	2.18	6.60	0.25
1-methylnaphthalene	0.03	0.03	0.47
2-methylnaphthalene	0.03	0.04	0.43
n-C14	2.71	2.65	0.51
n-C15	0.23	2.09	0.10

Figure 2-TDS-GC/MS analysis of a selected hydrocarbon mixture (Tremblay et al, 2008)



Characterization of petroleum-, synthetic-, and biomass-based fuel headspace vapor
Based on recent military and civilian interest in synthetic and biologically-based fuels a comparison of headspace vapor components from commercially available and recently developed fuel types was conducted using gas chromatography and mass spectrometry. Rodent inhalation exposures described earlier, utilizing aerosolized Jet Propulsion Fuel 8 (JP-8) and a synthetic Fischer-Tropsch (FT) fuel (S-8), revealed notable differences in the aerosol and vapor phase characteristics of the chamber exposure atmospheres. The S-8 fuel was found to be devoid of aromatic content, with the mass replaced by lighter hydrocarbons (C_8 - C_{10}), such as n-alkanes and branched aliphatics. In addition to JP-8 and S-8, headspace analysis was also conducted for a 50:50 blend of JP-8:S-8, petroleum-based diesel fuel, bio-diesel, and a biomass (bio-oil) fuel developed at the University of Georgia. Studies with a bio-fuel blend of biodiesel and the wood chip bio-oil contained quantifiable levels of toluene and benzene, as well as, components specific to wood, such as Alpha pinene, 2-beta pinene, Limonene, beta-Terpinene, Camphene, 2-methyl furan, and 2,5-dimethyl furan. In particular, components derived from oils and polymers of plant species may lead to novel exposures compared to petroleum based diesel fuels. This was the first work to compare headspace vapor composition between petroleum-based and synthetic jet fuels, and petroleum-based and bio-mass derived diesel fuels (Figure 3). *(Kendrick et al, 2008)*

Figure 3. Comparison of petroleum-based and synthetic-based fuel headspace

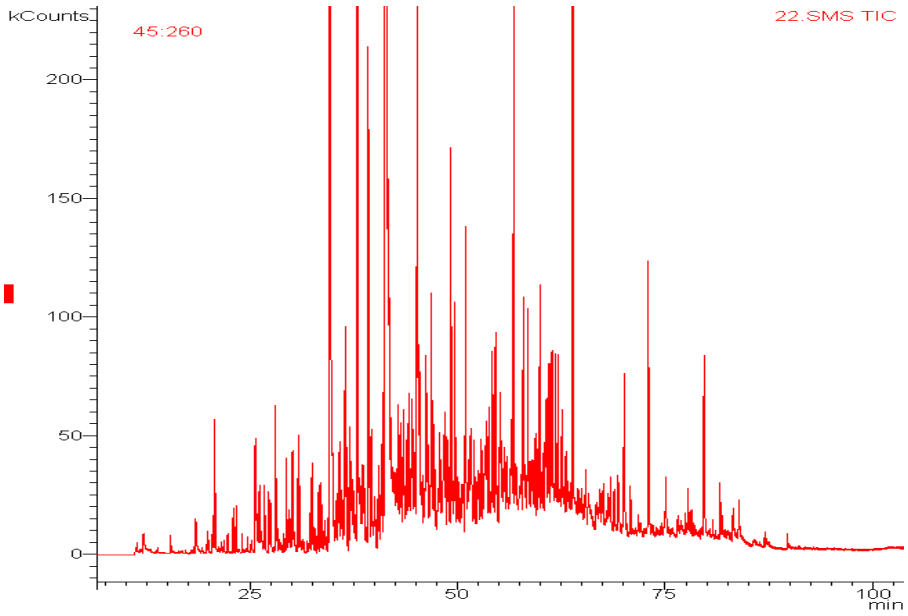


Compound Peak Identification				
A: toluene	B: octane	C: 4-methyloctane	D: o-xylene	E: nonane
F: 3-methylnonane	G: decane	H: 1,2,4-trimethylbenzene	I: undecane	J: dodecane

Improve our Tissue Characterization Method, in Support of PBPK Model Development

Our Solid Phase Micro-Extraction-GC/MS (SPME-GC/MS) method (FY-06), originally developed to quantify hydrocarbons in animal tissues represented a step forward in analysis of tissue concentrations of jet fuel constituents. In particular, the detection limit for many hydrocarbons was increased by a factor of 1000 with limits of quanitification as low as 1.9 ng/ml or g. During FY07, the method was optimized to shorten analysis time from about 110 min to about 70 min, effectively increasing our efficiency in processing and analyzing tissue samples collected during animal exposures that support model development. A major accomplishment of FY07 was the development of a method suitable to analyze hydrocarbon components in fat. Fat is difficult to analyze by SPME because its high lipophilic content interacts with the SPME fiber. Also preparation of accurate calibration standards was impossible, until this new fat analysis method was developed. Earlier calibration spikes did not incorporate well with the fat. The tissue method was applied to blood, fat, liver, lung, and brain and provided data for PBPK model development.

Figure 4. SPME-GC/MS Chromatogram of JP-8 jet fuel in blood following inhalation exposure. Data was used for determination of total concentration and individual constituent concentrations.



Understand the Metabolism and Elimination of Aviation Fuel Hydrocarbon Markers

Excretion of parent compounds (non-metabolized):
From the S-8 exposure, feces and urine were collected from four rats during the 48hrs following exposure. Feces samples were collected at 24hrs and 48hrs. No fecal matter was present at 6hrs. Urine samples were collected at 6hrs, 24hrs and 48hrs. Control samples were also collected from non-exposed rats. Approximately 50 hydrocarbons

were quantified in the collected samples and the data converted to rate (ng / hour) using sample mass and duration. Analytical methods used were similar to that used for the other collected tissues (presented in FY07 annual report). Significant amounts of higher molecular weight hydrocarbons (C12 to C15) were found in the feces of exposed rats. Excretion rates were found to correspond to the loss rate of these same compounds in fat.

Excretion of fuel metabolites:
As part of the analysis for parent compounds, potential metabolites were qualitatively identified and concentration measured on a relative scale. This first approach step provided a list of potential fuel metabolites of interest for which chemical standards were purchased in early year 3. Several of these metabolites were also measured and tracked in tissues. Identifications of hydrocarbon metabolite structures suggest involvement of hepatic cytochrome P450 system enzymes; which is supported in the literature. The burden of metabolites appeared to be greatest in the liver compared to other tissues. In separate inhalation exposures, n-alkane metabolites were also found in liver and lung of rats exposed to a mixture of n-alkanes (n-C10 to n-C15). An understanding of the excretion of select hydrocarbons and metabolites informed development of the PBPK model for aviation fuels. Selected data are presented in Tables 3 and 4.

Table 3. Fuel metabolite concentrations (ng g^{-1}) in liver, feces and urine from S-8 exposure and blood, lung and liver from designed mixture exposure (C10 to C15). Metabolites in bold quantified using authentic standards (error 20%). Concentration extrapolated for others (error 20-50%). Empty cells are below detection limits. (Tremblay et al, 2009)

	S-8			Custom Mixture		
	Liver (t=0h)	Feces (0-24h)	Urine (0-24h)	Blood (t= 0h)	Lung (t= 0h)	Liver (t= 0h)
2-octanone			4.9			
2,6-dimethyl-2-heptanol	11.7	487	43.1			
2-octanol			2.6			
3-methyl-3-octanol	7.2	666	37.8			
1-octanol			12.8			
2-nonanone	14.1					
4-nonanol		650.8				
3-nonanol	45.9	4730.7	49.7			
2-nonanol	38.6	945.4	150.3			
branched-C9-OH-A		581.8	9.1			
branched-C9-OH-B	4.6	1412.2	22.1			
3-methyl-3-nonanol	5.8	998				
3-decanone		79.5				8.3
2-decanone		308.4		17.6	15.5	31.5
4-decanol						36.2
3-decanol		2341.9	14.3			
branched-C10-OH-A		991.5	3			

branched-C10-OH-B		296.2	0.8			
2-decanol		542.6	11.9			
branched-C10-OH-C		363	1.2			
branched-C10-OH-D		334.6	1.2			
3-methyl-3-decanol		501.1				
branched-C11-OH-A		182.6				
3-undecanone		24.1				
2-undecanone				20.2	122.5	25
branched-C11-OH-B		314.3				
branched-C11-OH-C		145.6				
branched-C11-OH-D		59.7				
branched-C11-OH-E		169.5				
2-undecanol				32.5		166.4
3-methyl-3-undecanol		95.1				
2-dodecanone			0.2	8.6	241.8	9.7
2-dodecanol				33.2	514.5	
3-methyl-3-dodecanol		14.3				
2-tridecanol					190.2	
2-tetradecanol					41.4	

Table 4. Amount of fuel components excreted (ng) in feces and urine (0-48 hour) along with exposure concentrations (mg m^{-3}). Bold lines separates chemical into isomer groups. Error estimated to 20 %. Empty cells are values below detection limits. (Tremblay et al, 2009)

	Excreted 0-48h		Exposure concentration	Total excreted / exposure concentration (mL)
	Feces	Urine		
2-methylhexane	27.5	5.8		
heptane	25			
2-methylheptane		0.9	10.4	0.09
4-methylheptane	145.3	209.2	4.4	80.17
3-methylheptane	49.6	6.9	15.1	3.75
octane	129.7	70.7	51.4	3.9
2,5-dimethylheptane	77.3	14.4	24.2	3.78
3,3-dimethylheptane		7.4	3.7	2
2,3-dimethylheptane		2.8	6.8	0.41
2-methyloctane		0.8	92.3	0.01
3,4-dimethylheptane		11	8.4	1.31
3-methyloctane	41.6	4.4	58.6	0.78
3,3-diethylpentane	27.3	4.7	1.8	17.84
nonane	20.5	2.4	59.7	0.38
2,2-dimethyloctane	16.3		6.8	2.41
Continued.	Excreted 0-48h		Exposure concentration	Total excreted / exposure concentration (mL)

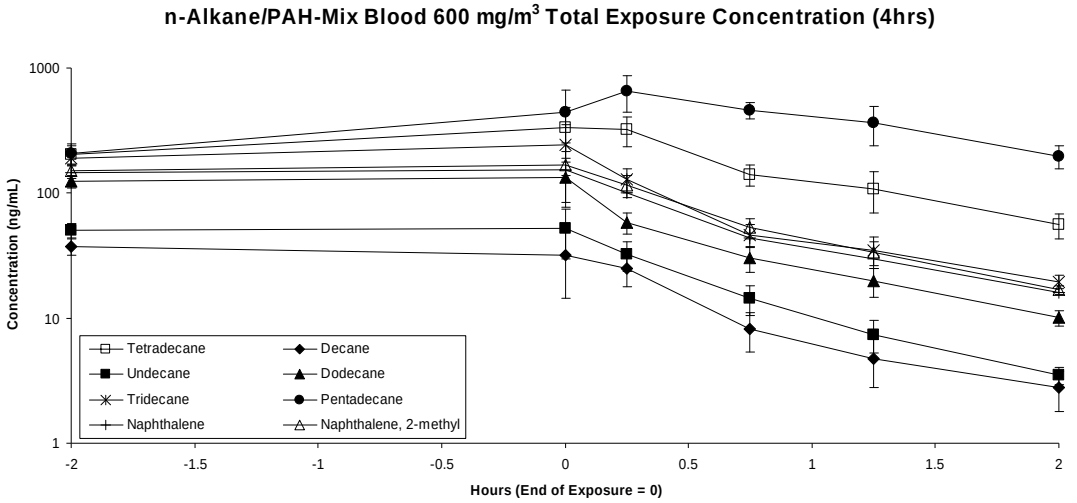
2,3-dimethyloctane	8.9	0.5	2.9	3.28
3,3-dimethyloctane	49.9	96	14.9	9.77
2-methylnonane	22.4	4	27.4	0.96
3-ethyloctane	33.3	2	5.7	6.24
3-methylnonane	28.6		28.2	1.01
decane	50	21.1	41	1.73
4-methyldecane	54	14	12.2	5.59
2-methyldecane	14.6	1.2	13.1	1.21
3-methyldecane	14.1	1.2	14.5	1.05
undecane	73.9	40.9	25	4.6
dodecane	88.1	18.8	16	6.69
tridecane	129.1	9.5	10.9	12.74
tetradecane	316.3	10.9	11.7	27.97
pentadecane	231.1	17.5	3.4	72.47

Animal Exposures

Exposures to a Custom Mixture of Eight Hydrocarbons, Collection of Tissue Data

Fischer-344 rats approximately 200 g were exposed via 4 hour inhalation to an 8 hydrocarbon mixture (n-decane, n-undecane, n-dodecane, n-tridecane, n-tetradecane, n-pentadecane, 2-methylnaphthalene, naphthalene) in combined aerosol and vapor form in 3 separate experiments (1269, 661, 565 mg/m^3). For each run, 6 time points were collected by exposing 24 rats (4 rats per time point, plus 4 control rats/run). Each experiment consisted of three separate 4 hour exposures of 8 rats, to collect two time points/run. Lumped atmospheric samples were collected every 2 minutes via transfer line (200ºC) mounted on an Agilent GC/FID; in order to monitor stability of atmospheres during exposure. Identification and quantification of individual atmospheric hydrocarbons during each run utilized charcoal tubes collected during exposure (7 sets of 2 tubes per 4 hour exposure) for analysis via TDS-GC/MS. Tissues collected varied depending on exposure concentration and overarching goal of each study. For example, for the study at ~600 mg/m^3 total (aerosol+vapor) concentration, tissues were collected at 2 hours into exposure, 4 hours into exposure, 0.25 hours post exposure, 0.75 hours post exposure, 1.25 hours post exposure, and 2 hours post exposure to assess kinetics in specific tissues relevant to model development. In the case of fat studies (Table 1), time points were spread over 4 days. Tissues analyzed included blood, liver, lung, brain, and in specific cases fat. Figure 5 shows blood concentrations recorded for the ~600 mg/m^3 exposure. Overall, the data captures well the loading and unloading of select hydrocarbons in tissues and provided a large dataset to draw from for our modeling needs.

Figure 5. Selected Data (lines do not represent simulations)



Exposures to Complex Hydrocarbon Mixtures, JP-8, S-8, and 50:50 JP-8:S-8 blend.

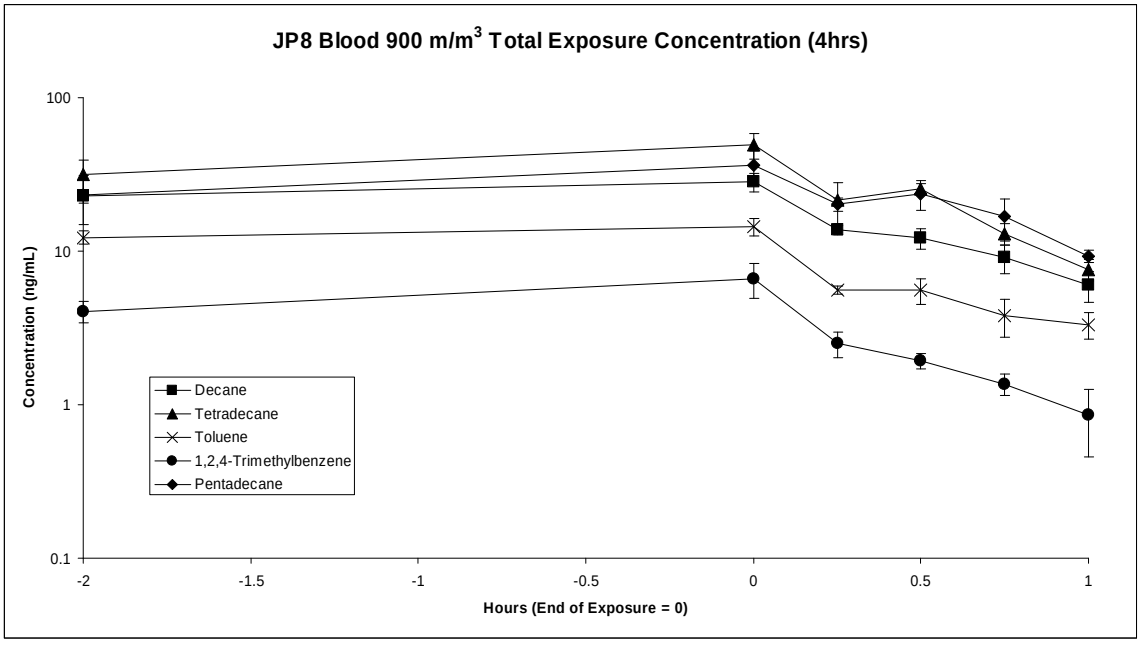
Male Fischer-344 rats approximately 200g were exposed 4 hours via nose-only inhalation to aerosolized fuel concentrations of 900, 1060, 200 mg/m3 for JP-8, S-8, and a 50:50 blend, respectively (Table 1). Exposures to JP-8, S-8, and blended JP-8:S-8 included collection of blood, liver, fat, lungs, and brain for pharmacokinetic analysis. To investigate potential metabolic interactions and clearance behavior of hydrocarbons in the synthetic fuel, a metabolism cage study extending 48 hours post-exposure was also conducted; with collection of feces and urine for biomarker analysis. Tissue collection time-points were designed to maximize our ability to adequately describe kinetic profiles of selected marker chemicals in tissues of interest (Table 1). All data points represented the mean and standard deviation of 4 rats.

Fuel inhalation experiments consisted of separate 4 hour exposures of 8 rats each, collecting 2 time points per exposure. 6 time points were collected for most full scale exposures, such that 28 rats (including 4 controls), were analyzed per exposure chemical. This experimental design yielded 120 exposed tissues and 20 control tissues. Tissue analysis included collection of approximately 6500 data points from these exposed animals (120 exposed samples * 54 compounds tracked in tissue) for each exposure conducted (Figures 1-4). All samples were stored at -80C until they were analyzed on a Varian SPME-GC/MS (Varian, Inc. Palo Alto, CA), with Combi-Pal robotic arm, under the method previously developed in Campbell and Fisher, 2007 and modified as per the 2007-2008 AFOSR annual report and described above.

Chamber atmospheres were monitored via 3 methods. As opposed to the custom mixture of 8 chemicals described earlier, where total hydrocarbon concentration (THC) was monitored via adsorbent tubes, here the exposure atmosphere concentration and stability were monitored via transfer line (200ºC) mounted on an Agilent 6890 GC/FID (Agilent Technologies, Santa Clara, CA) equipped with gas-sampling valve. The adsorbent tubes were collected in ~40 minute intervals for analysis via TDS-GC/MS. Separate gas phase and aerosol phase concentrations for a series of 63 fuel constituents of interest were obtained from these tubes, allowing calculation of aerosol and vapor percent

composition for each constituent relative to both the known fraction of JP-8, S-8, 50/50 blend, and to the total fuel concentration. Both types of data are necessary for PBPK model development. Approximately 1500 concentration data points from the chamber atmosphere were collected per exposure for JP-8, and S-8, with slightly less for blended JP-8:S-8. A 7-stage cascade impactor (0.25-5 µm) was used to assess aerosol droplet size and distribution (Intox Products, Moriarty, NM). Data analysis utilized the manufacturer's specific cascade impactor data reduction program (Intox Products, Moriarty, NM) and the Multiple-Path Particulate Dosimetry model (MPPD2, Hamner Inst., RTP, NC). Selected blood concentration data is presented (Figure 6) and includes both aromatic and n-alkanes that were tracked in the rodent.

Figure 6. Selected Data (lines do not represent simulations)



Exposures to n-Tetradecane and n-Octane as PBPK Model Marker Chemicals

Fischer-344 rats approximately 200 g were exposed via 4 hour nose-only inhalation to n-tetradecane. Investigation of potential metabolic interactions and method development requirements related to our understanding of aerosol deposition and uptake of a single high molecular weight fuel marker necessitated exposure to n-tetradecane. Exposures were conducted at approximately the concentration of n-tetradecane in the 8 hydrocarbon mix (89 mg/m^3) during the previous lower concentration 8 hydrocarbon custom mixture studies. Exposure methods were identical to those reported for the 8 hydrocarbon mixture. Results of the study informed model development for higher molecular weight hydrocarbon constituents of jet fuel, especially in the characterization of the lung and development of the fuel "lumping" strategy described later. This is the first such

exposure to n-tetradecane in either aerosol or vapor form and represents the only pharmacokinetic dataset with this chemical.

In a separate study, Fischer-344 rats approximately 200 g were exposed via 2 hour nose-only inhalation to n-octane vapors at approximate concentrations 5000ppm, 1000ppm, 100ppm. The exposure utilized a fritted-glass bubbler with inline glass wool scrubber to ensure a vapor-only atmosphere. Total hydrocarbon concentration was assessed via GC/FID using the existing transfer line and gas-sampling valve apparatus. Blood, liver, lungs, fat, and brain were collected for the time course study at 1000ppm. Blood, brain, and lung were collected at the end of exposure time point for 100 and 5000ppm. Exposures to C8 vapor, for kinetic data collection, have not been published since the early 1990s, and did not include multiple time points. As no sufficient animal kinetic data was currently available for development and validation of PBPK models for this chemical, it was necessary to fill this data gap through *in vivo* exposure and tissue collection. The collected tissue data was useful in development of an n-octane PBPK model that can be easily modified for simulation of kinetic behavior associated with other low molecular weight n-alkanes present in aviation fuels. Tissue data collected from the n-tetradecane and n-octane studies were used in model development, informing the higher molecular weight (high percentage aerosol) and lower molecular weight (predominately vapor phase) chemical modeling efforts, respectively.

Figure 7. Selected Data (lines do not represent simulations)

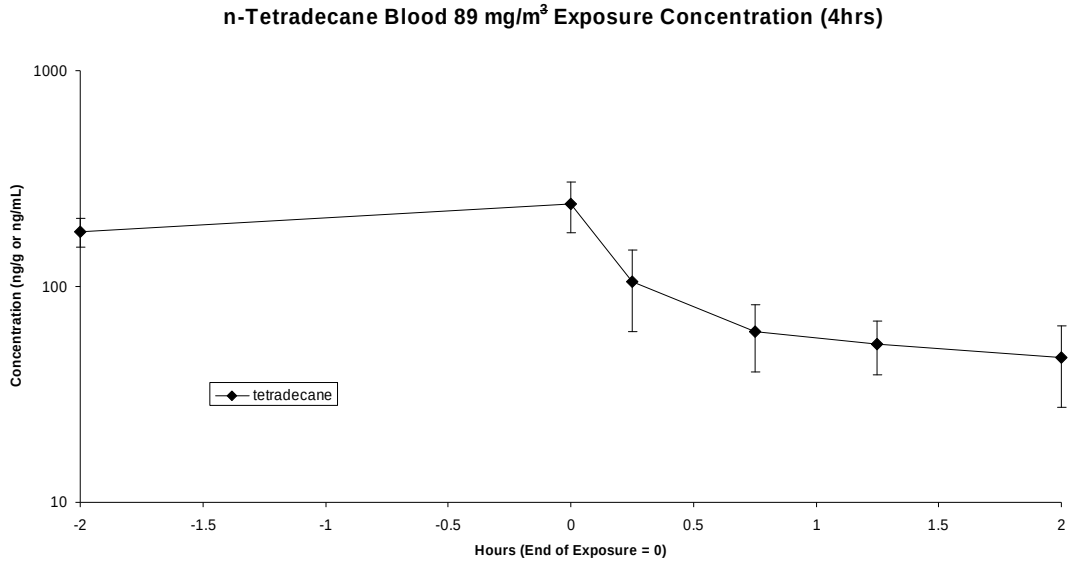
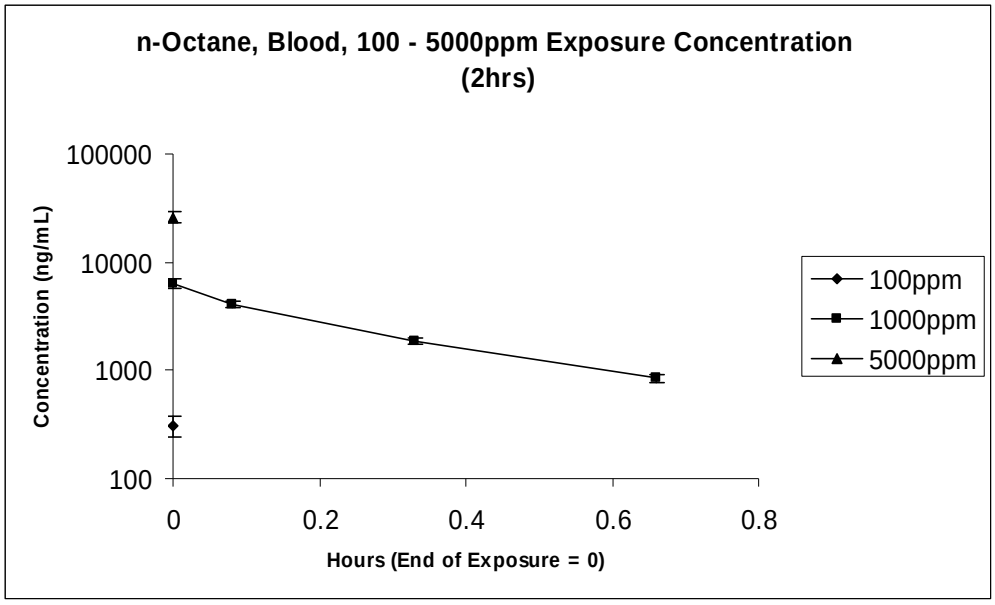


Figure 8. Selected Data (lines do not represent simulations)



Model Development

Development a PBPK Model for Aviation Fuels

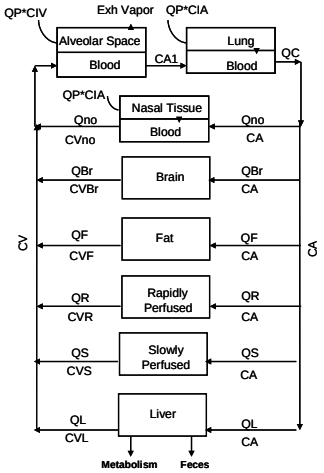
The collected kinetic data provides a large dataset to draw from for PBPK model development at University of Georgia and for use by collaborators at Wright Patterson Air Force Base. In developing a PBPK modeling framework to address complex HC mixtures (JP-8, S-8, etc) we note that higher molecular weight species in both fuels predominate in the aerosol phase while species of lower molecular weight, including some select n-alkanes, isoalkanes, and aromatics, remain predominately in the vapor-phase. Acknowledgment of the differences in chemical partitioning in the exposure atmosphere required development of a novel PBPK modeling framework to assess droplet uptake and distribution when the exposure methodology includes aerosol generation. Additionally, these differences influenced selection of "marker" chemicals for the development of chemical sub-models. Marker chemicals that were selected include: toluene, m-xylene, ethylbenzene, n-octane, n-decane, and n-tetradecane; with the aromatics and n-octane present predominately in the vapor phase and higher molecular weight n-alkanes present predominately in the aerosol phase. The UGA TDS-GC/MS method for atmospheric characterization (Tremblay et al, in press) provides the capability to assess constituent percent aerosol and vapor concentration relative to the total mixture. The Multiple-Path Particulate Dosimetry (MPPD2) software package (Hamner Institutes, RTP, NC), allows prediction of the percent inhaled droplets that deposit in select regions of the upper respiratory tract and the lung. With these factors in mind, the ACSLX modeling software (Aegis Technologies, Huntsville, AL) was used to develop compartments for the upper and lower respiratory tract; describing the uptake and distribution of aerosolized hydrocarbon droplets. A gas-exchange compartment was also added, to account for the percentage of chemical present as vapor during an exposure. Thus, both aerosol droplet and vapor phase concentrations of total fuel and individual constituents were accounted for. The flexibility of the model structure also allowed

simulations of 100% vapor exposure atmospheres; such that comparisons to our existing vapor-only jet fuel datasets were possible. Additional compartments for liver, brain, fat, slowly and richly perfused tissues were added and connected by blood flow, to account for metabolism and the storage depot for lipohilic chemicals (fat). Compartment physiological parameters (blood flows, tissue volumes) were collected from the literature. Model equations were written to describe adsorption, distribution, metabolism, and excretion of fuel constituents. Depending on the submodel used and the chemical of interest, either perfusion or diffusion-limited equations were written to describe a particular tissue compartment. Of note, most chemicals were assumed to interact competitively at the common site of metabolism, the P450 enzyme (liver compartment). As no metabolic activity has been reported for higher molecular weight constituents, such as n-tetradecane, no metabolism was assumed. During simulated fuel exposures, the exposure concentration of a chemical of interest was modeled with other quantified chemicals and defined chemical "lumps" of unspeciated fuel constituents. Lump parameters were averaged from representative chemical markers that were assigned to the particular lump. Metabolic parameters for the lumps were fit. The final PBPK model included three lumps that were constructed from data collected during animal exposures, to account for the total fuel exposure concentration. The three lumps represented the aromatic/branched alkane low molecular weight fraction (Figure 9a), an intermediate molecular weight fraction (Figure 9b), and a high molecular weight lump.

Simulations of blood toluene concentration were conducted and compared to data collected following exposure to aerosolized JP-8 (total exposure concentration 900 mg/m^3) and to model simulations of vaporized JP-8 (total exposure concentration 2500 mg/m^3) collected during a previous grant cycle. Good agreement was seen in simulations of data from both the aerosolized fuel exposure and the vaporized fuel exposure (Figure 10a,b), providing an indication of the applicability of the model for use in both types of fuel exposures. Simulated n-C10 blood concentrations were compared to tissue data collected at 1200ppm n-C10 (Perleberg et al, 2004) (Figure 10c) and vaporized JP-8 (total exposure concentration 2500 mg/m^3) (Figure 10d) collected during a previous grant cycle, as well as to aerosolized JP-8 exposure data (total exposure concentration 900 mg/m^3) (Figure 10e) and aerosolized S-8 exposure data (total exposure concentration 1060 mg/m^3) (Figure 10f) collected in this grant cycle. Comparison of the data from individual chemical exposure, vaporized JP-8 exposure, and aerosolized exposure to JP-8 and S-8 highlight the utility of this PBPK model to predict data sets collected across multiple exposure types. A manuscript describing model development and application is currently in development, prior to submission for publication in 2010.

Figure 9. Example Model Schematics

a)



b)

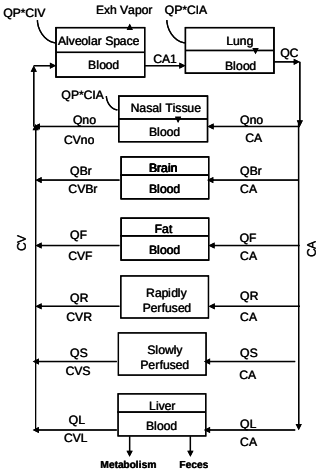
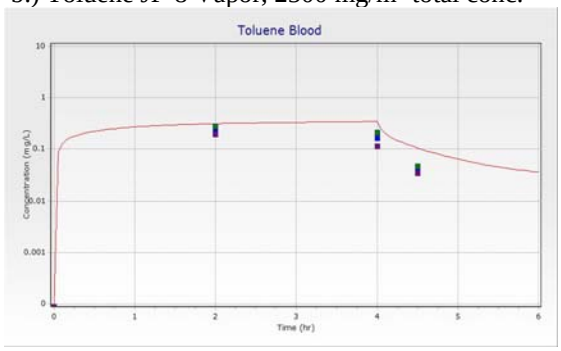


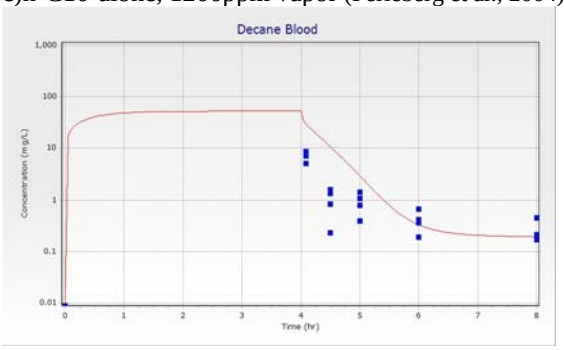
Figure 10. Example PBPK Model Simulations of Toluene and n-C10 in Blood

a.) Toluene JP-8 Aerosol (900 mg/m^3) total conc.

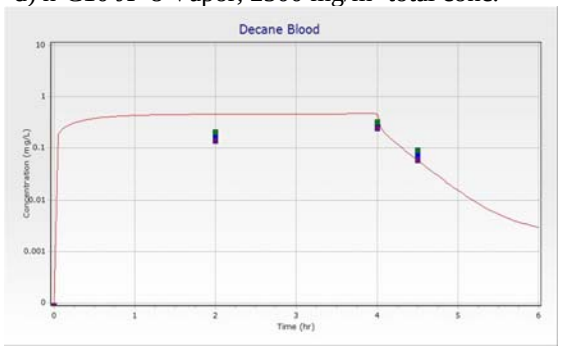
b.) Toluene JP-8 Vapor, 2500 mg/m^3 total conc.



c)n-C10-alone, 1200ppm vapor (Perleberg et al., 2004)

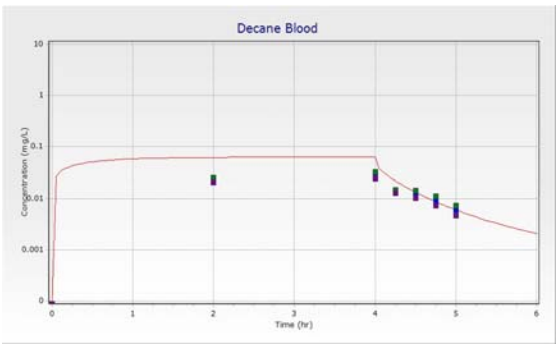


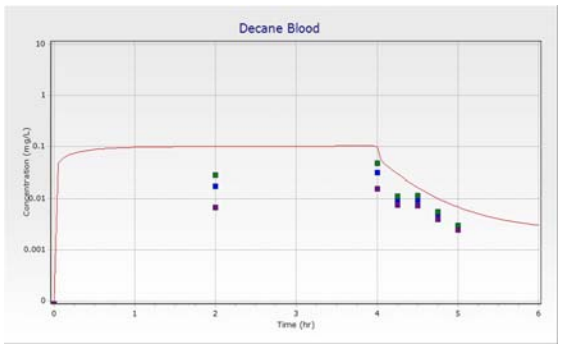
d) n-C10 JP-8 Vapor, 2500 mg/m^3 total conc.



e) n-C10 Aerosol JP-8 (900 mg/m^3) total conc.

f) n-C10 S-8 Aerosol (1060 mg/m^3) total conc.



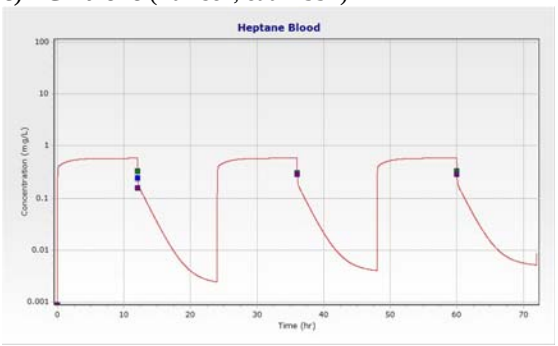


Development of PBPK Models for n-Alkane Hydrocarbon Mixture Constituents

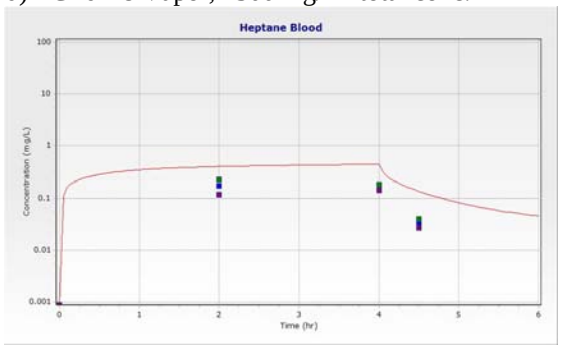
Preliminary PBPK models were developed to investigate the kinetic behavior of n-alkanes (C6-C15) present in common complex hydrocarbon mixtures. Model structures were the same as those developed for the larger fuel PBPK model and adapted for the additional n-alkanes of interest. These n-alkanes may eventually become marker constituents for an updated JP-8 model, however, due to a current lack of sufficient individual chemical exposure data in the literature to validate some of these individual constituents, inclusion into the current JP-8 model was not been done. Use of our custom n-alkane/PAH 8 component mixture may provide sufficient data for this purpose. Example model simulations are presented below (Figure 11). In the case of n-heptane, the preliminary model is shown compared to a dataset from (Zahlsen et al., 1992) at 100ppm/12hr-day/3-days (Figure 11a) and compared to data collection following a 4-hr/day exposure to JP-8 at a total fuel concentration of 2500 mg/m^3 (Figure 11b). A preliminary PBPK model for n-nonane, adapted from our JP-8 model structure, is shown compared to data from a 4-hr/day exposure at 500ppm vapor (Robinson and Merrill, 2007) and data from an 8-hr/day exposure to 3600 ppm (Nilsen et al., 1988) (Figure 11c,d).

Figure 11. Example PBPK Model Simulations of n-C7 and n-C9 in Blood

c)n-C7-alone (Zahlsen, et al 1992)

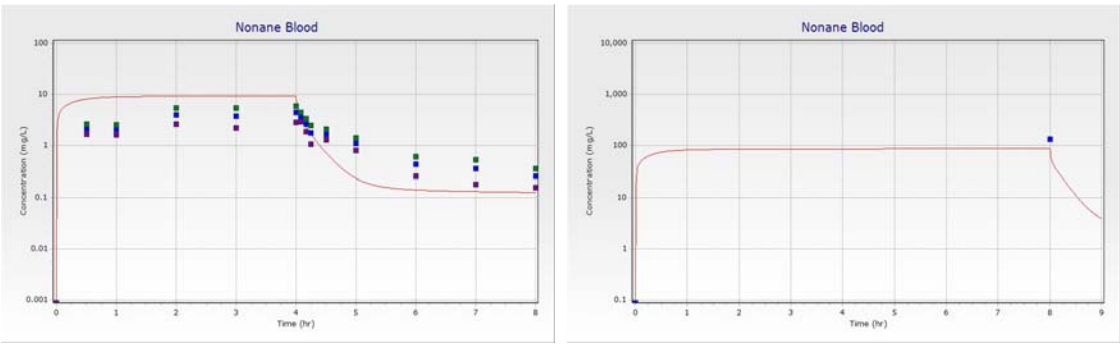


d)n-C7 JP-8 Vapor, 2500 mg/m^3 total conc.



c)n-C9-500ppm (Robinson and Merrill, 2007)

d)n-C9 3600ppm (Nilsen et al, 1988)



Closing Data Gaps

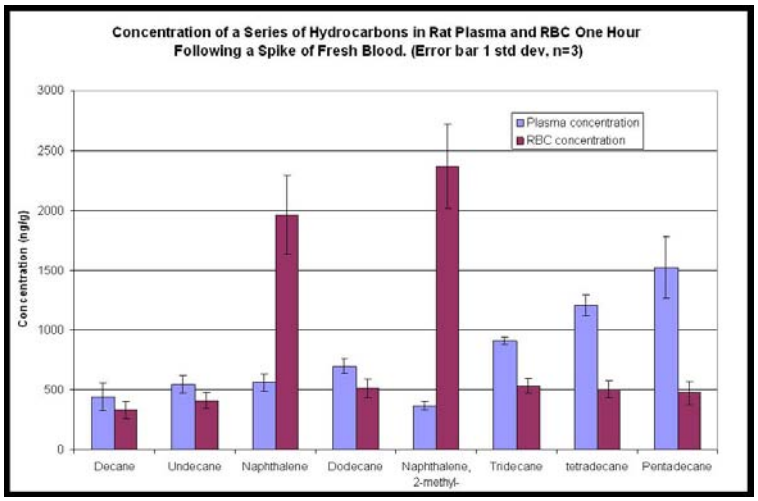
Investigation of Potential Blood Cell Binding by Aviation Fuel Hydrocarbons

Heparinized rat whole blood was spiked with a solution of the 8 hydrocarbon mix to investigate the theoretical concept that jet fuel related hydrocarbons will partition into different blood compartments, cells vs. plasma, depending on lipophilicity and molecular weight, and that this may alter the kinetic profiles of these compounds. Following a study design that allowed for time course analysis of the binding, the separated plasma and red blood cell layers were quantified by SPME-GC/MS.

1.5 mL of heparinized rat whole blood was dispensed into microcentrifuge vials where it was spiked with 10 uL of a dilution of the 8 hydrocarbon mix in hexane. The samples were allowed to come to equilibrium across 20, 60, 120, 180, 240 minutes. Samples were centrifuged 15 minutes at 1300 rpm/min. The separated plasma and red blood cell layers were pipetted into separate scintillation vials for SPME-GC/MS analysis. 3mL of salt water was added to each vial before analysis. Compositional analysis was via comparison to simultaneously prepared chemical standard curves.

Partitioning appeared to be complete after one to two hours. Initial studies with freshly collected rat blood (less than 24h old) showed that naphthalene and 2-methylnapthalene were enriched by factors of 4 to 6 in the red blood cells versus the plasma. Lower molecular weight alkanes (decane to dodecane) were found to be partition equally or remain slightly enriched in the plasma while the heavier alkanes (tridecane to pentadecane) were enriched by factors of 2 to 3 in the plasma (Figure 12). The same experiment was repeated in more detail using fresh but older rat blood (4 to 10 days old) obtained from a commercial supplier (Innovative Research, Southfield, MI). Similar trends were observed for the naphthalenes and tridecane to pentadecane, but the enrichment differences did not appear to be as significant when compared to the first experiment. The differences in partitioning behavior between cells and plasma across the n-alkane series and the naphthenic constituents may have implications for in vivo kinetic behavior.

Figure 12. Results of Binding Experiments



Fill Data Gaps in the Partition Coefficient (PC) Literature Related to PBPK Model Development. Specifically, Collect Lung PC Values for Marker Chemicals Where Data is Lacking in the Current Literature.

Partition coefficients were determined as a ratio of chemical in tissue versus chemical in air for select fuel hydrocarbons. Chemicals of interest included: n-octane, n-nonane, 1,2,4-trimethylbenzene, and 4-isopropyltoluene. Each chemical partition coefficient was first determined for liver and fat to compare to existing data (if available) within the literature for verification of experimental and analytical methods.

Experiments utilized the vial equilibration technique (Smith et al., 2005). Tissues were weighed, minced, and then smeared into 10mL round-bottomed head space vials that were subsequently sealed. An equal number of reference vials were prepared and tested in the same manner. All vials were then warmed to body temperature so that an equal volume of air could be removed from the headspace and replaced with air from a Tedlar bag of known concentration. Vials were then lightly shaken and warmed at body temperature for three hours. Finally, the concentration of each vial was determined by manual injection into an Agilent 6890 GC/FID (Agilent Technologies, Santa Clara, CA). The GC/FID was equipped with an HP-5 column (10 m × 0.53 mm × 2.65 μm). The injector, FID, and oven temperatures were 230◦C, 270◦C, and 140◦C, respectively. The helium, hydrogen, and air flows were 2.3, 23, and 210 ml/min, respectively, with a split of 2.2 ml/min. The differences between reference vials and sample vials were used to determine the affinity of the chemical for the tissue tested.

Lung tissue partition coefficients were determined for octane, nonane, 1,2,4-trimethylbenzene. Brain, fat, kidney, and liver partition coefficients were determined for 4-isopropyltoluene. Appropriate data for each chemical was lacking in the literature and required for model development. Results were first scrutinized for precision and accuracy before they were determined acceptable data. Partition coefficients for octane, nonane, and 1,2,4-trimethylbenzene in lung tissue were 87.267, 121.104, and 184.052, respectively. Tissue partition coefficients have previously been reported for other tissues (including fat and liver) that were collected concurrently. As our data for these tissues

was in agreement with published values, the new partition coefficients for lung were deemed acceptable for use in PBPK model development. Table 5 provides a summary of data with comparison values from published sources where applicable.

Table 5. Partition Coefficients

Chemical	Tissue	PC-Obtained (Tissue:air)	PC-Literature (Tissue:air)	Reference
n-Octane	Liver	11.04	6.01	Smith, 2005
	Lung	87.27	n/a	
	Fat	668.2	771.9	Smith, 2005
	Brain	varied	4.38	Smith, 2005
n-Nonane	Liver	11.13	11.32 (a) 6.64 (b)	(a) Smith, 2005 (b) Robinson, 2000
	Lung	121.1	n/a	
	Fat	2321.85	1588.2 (a) 1254 (b)	(a) Smith, 2005 (b) Robinson, 2000
	Brain	25.86	22.3 (a) 7.13 (b)	(a) Smith, 2005 (b) Robinson, 2000
1,2,4-Trimethylbenzene	Liver	279.12	374.44	Hissink 2007 *
	Lung	184.05	n/a	
	Fat	8198.89	9279.6	Hissink 2007 *
	Brain	varied	374.44	Hissink 2007 *
4-Isopropyltoluene	Liver	72.33	n/a	
	Kidney	26.22	n/a	
	Fat	591.3	n/a	
	Brain	268.65	n/a	

*Value originally published as Tissue:Blood, presented here as Tissue:Air for continuity. Multiplication of the Tissue:Blood PC by the Blood:Air PC cited in the work, yields a Tissue:Air PC value.

Determine Partition Coefficients for a Series of Isomers of n-Nonane

The lack of experimentally determined partition coefficients (PC) for fuel related chemicals represents a barrier to the development of PBPK models (Payne and Kenny, 2002). We report the partition coefficients of Nonane and five isomers of nonane, 2,2,4-trimethylhexane, 3-methyloctane, 4-ethylheptane, 2,3-dimethylheptane, 2,2,4-trimethylhexane and 2,2,4,4-tetramethylpentane. The isomers for the determination of PCs were selected based on their octanol water (logKow) value which will eventually be used for the development of PBPK model for hydrocarbons present in jet fuel.
The headspace vial equilibration method is a commonly used method for determination of PCs (Gargas et al., 1989, Kumarathasan et al., 1998, Sato and Nakajima, 1979, Smith et al., 2005). Three-liter (3L) SKC Tedlar Sample Bags were filled to 80% capacity with HEPA+charcoal filtered room air and spike with known concentrations of each chemical using the equation:

$$\text{Vol (ml)} = \frac{\text{ppm x Specific Gravity of the chemical x molecular weight}}{\text{Molar Volume x } 10^6}$$

About 0.5 g of liver, 1 g of muscle, 0.4 g of lung, 0.1 g of brain, 0.05 g of fat were minced in the crucible and smeared in 10 ml glass vials. 0.75 ml of whole blood was used. Reference vials for each set of sample tissue and blood were also prepared. Vials were vortexed at 37^{o}C for 20-30 minutes, and then 1 ml of gas from the standard bag was added to each vial, after removing 1 ml of air from the vial using a gas tight syringe. Vials were incubated in the vortex for 3 hours (blood, liver, lung and muscle) or 4 hours (fat and brain) before 0.5 ml of the headspace vapor was injected into an Agilent Technology 6890 N series II gas chromatograph equipped with a DB5 megabore capillary column (15.0 m x 530 µm x 1.5 µm).
PCs were calculated using the equation found in Gargas *et al*. (1989),

$$P_i = \frac{C_{ref}\ (V_{vial}) - C_i\ (V_{vial}\text{-}V_i)}{C_i\ V_i}$$

Where, *Pi* = partition coefficient. *Ci* = concentration of n-alkane vapor contained in headspace of reference vial, V*vial* = volume of reference vial (11.3 ml headspace vial). *Ci* = concentration of n-alkane in headspace of test vial, and V*i* = volume of tissue/blood in test vial. Five replicates of tissue and sample were prepared for the PCs determination.

n-Nonane was determined to have the highest solubility in fat followed by brain. Muscle was the least soluble (Table 6). PCs of liver and lung were very similar. The tissue:air PC of chemical in all tissues decreased with increased branching of the isomer. PC values for blood and tissues of most isomers were found to follow the octanol:water coefficient value. Of the chemicals studied, the only reported experimental value from the head space vial method present in the literature is for nonane (Robinson, 2000)(Smith et al., 2005). No data on the tissue: air and blood: air PCs for the isomers have been reported. Our n-nonane PC values for tissue:air were consistent with one of either Smith *et al*., 2005 or Robinson, 2000 except for blood:air and fat:air, which were higher than that reported by both. Smith *et al*. reported nonane PCs for blood:air, liver:air, fat:air, muscle:air and brain:air as 5.8, 11.3, 1588, 4.7, and 22.3, respectively using thawed frozen tissue and blood. Robinson, 2000 reported these values as 5.1, 6.6, 1254, 7.1, and 25.9, respectively using fresh tissue and blood. Our experimentally derived PC values for the above mentioned tissues were 10.5, 15.83, 2206.14, 7.24 and 32.05 respectively. The straight chain n-nonane had the highest PC in most cases and the trend was clearly observed in the most lipophilic tissues; fat and brain. The coefficient of variation (CV) ranged from 1 to 15% across all isomers. The CVs of n-nonane for the Smith et al PCs were 32%, 30%, 14%, 15% and 25% for fat, blood, brain, liver and muscle respectively. In comparison, the CV of our PCs for n-nonane ranged from 4% (fat) to 10% (liver).
Table 6. n-Alkane Tissue:air and Blood:air partition coefficients (mean (CV))

Chemical/PC (SE)	Blood:air	Liver:air	Fat:air	Brain:air	Lung:air	Muscle:air
Nonane	10.5 (0.05)	15.83 (0.10)	2206.14 (0.04)	32.05 (0.07)	13.01 (0.05)	7.24 (0.08)
3-methyloctane	9.35 (0.11)	13.18 (0.10)	1755.95 (0.03)	27.99 (0.09)	12.81 (0.06)	7.98 (0.05)
4-ethylheptane	13.56 (0.05)	11.73 (0.05)	1941.69 (0.03)	26.30 (0.07)	12.37 (0.09)	7.93 (0.07)
2,3-dimethylheptane	9.14 (0.08)	10.89 (0.10)	1663 (0.01)	24.07 (0.06)	14.87 (0.03)	5.46 (0.07)
2,2,4-trimethylhexane	5.79 (0.06)	4.48 (0.03)	790.77 (0.06)	17.52 (0.09)	4.83 (0.02)	4.11 (0.10)
2,2,4,4-tetramethylpentane	4.24 (0.08)	4.92 (0.09)	698.55 (0.05)	11.53 (0.05)	3.14 (0.15)	2.64 (0.15)

Upcoming Studies

This is the final report for this grant cycle. Continued work will be in refinement and publication of PBPK models for jet fuels, n-alkanes, iso-alkanes, the n-alkane/PAH custom mixture, and select aromatics, as well as publication and use of S-8 metabolism/kinetic data, and partition coefficient methods/data for the C9 (n-nonane) isomers.

References

Gargas M, Burgess R, Voisard D, Cason G, Andersen M. (1989). Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. Toxicol Appl Pharmacol, 98, 87-99.

Kumarathasan P, Otson R, Chu I. (1998). Application of an automated HS-GC method in partition coefficient determination for xylenes and ethylbenzene in rat tissues. Chemosphere, 37, 159-178.

Nilsen O, Haugen O, Zahlsen K, Halgunset J, Helseth A, Aarset H, Eide I. (1988). Toxicity of n-C9 to n-C13 alkanes in the rat on short term inhalation. Pharmacol Toxicol, 62, 259-266.

Payne M, Kenny L. (2002). Comparison of models for the estimation of biological partition coefficients. J Toxicol Environ Health Part A, 65, 897-931.

Perleberg U, Keys D, Fisher J. (2004). Development of a physiologically based pharmacokinetic model for decane, a constituent of jet propellent-8. Inhalation Toxicol, 16, 771-783.

Robinson P. (2000). Pharmacokinetic Modeling of JP-8 Jet Fuel Components: I. Nonane and C9-C12 Aliphatic Components. Storming Media.

Robinson P, Merrill E. (2007). A Harmonized Physiologically-Based Pharmacokinetic Model for Nonane as a Component of Jet Fuel. Henry M. Jackson Foundation for the Advancement of Military Medicine. AFRL-RH-WP-TR-2008-0067. Wright-Patterson Air Force Base, OH:

Sato A, Nakajima T. (1979). Partition coefficients of some aromatic hydrocarbons and ketones in water, blood and oil. Br Med J, 36, 231.

Smith A, Campbell J, Keys D, Fisher J. (2005). Rat tissue and blood partition coefficients for n-alkanes (C8 to C12). Int J Toxicol, 24, 35-41.

Zahlsen K, Eide I, Nilsen A, Nilsen O. (1992). Inhalation kinetics of C6 to C10 aliphatic, aromatic and naphthenic hydrocarbons in rat after repeated exposures. Pharmacol Toxicol, 71, 144-149.